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The role of neutrophils and platelets on the development and enlargement of jaw cysts

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Abstract
Aim: The aim of this study was to compare the circulatory inflammatory cells in peripheral blood tests of patients with odontogenic cysts and healthy subjects and the effect of these markers between the development and enlargement of cysts.
Material and Methods: Ninety-eight patients with an odontogenic cyst in the maxillofacial region (Group 1) and 102 healthy subjects as control (Group 2) were included in the study. White blood cell count, neutrophil count, lymphocyte count, platelet count, neutrophil to lymphocyte ratio (NLR), mean platelet volume (MPV), and platelet to lymphocyte ratio (PLR) were compared between groups. In addition, the correlation of these markers with volume of the cysts was determined.
Results: Fifty-six radicular cysts, 34 dentigerous cysts, and 8 odontogenic keratocysts were observed in Group 1. Lymphocyte count, platelet count, and MPV were statistically higher in Group 2 ($p<0.05$). The optimum cut-off level for MPV was detected as 8.7 according to the ROC analysis (Sensitivity: 85, Specificity: 80). The median cyst volume was 4663 mm3 (min: 1213 max: 48553) and cyst volume was not different according to the type of the cyst ($p=0.063$). A positive and significant correlation between neutrophil count and PLR with cyst volume was observed ($p=0.023$, $p=0.007$)
Discussion: This study considered the effect of circulatory inflammatory cells on the pathogenesis of jaw cysts. Lower platelet counts and MPV may have an essential role in the development of cystic jaw lesions and higher circulating neutrophils may be associated with an expansion of the cysts.

Keywords
Odontogenic Cysts, Neutrophil, Platelet

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Introduction

Odontogenic jaw cysts are common pathological destructive lesions in the maxillofacial region and are composed of an epithelial tissue wall and fluid content [1]. The most common odontogenic cystic lesions are radicular cysts, dentigerous cysts, and odontogenic keratocysts. The odontogenic keratocyst, which has been termed previously as a "keratocystic odontogenic tumor", was reintroduced as a cystic lesion in the 2017 World Health Organization (WHO) classification [2]. The exact mechanism of growth and enlargement of cystic lesions is still controversy but it is reported that local immunological responses play a role in the pathogenesis of these lesions [3]. T-lymphocytes, macrophages, plasma cells, mast cells, and many other cytokines are associated with cellular immune response and the modulation of inflammatory process [4]. However, neutrophils, lymphocytes, and platelets (PLT) are key players in the systemic and local inflammatory response [5]. Mean platelet volume (MPV), neutrophil-to-lymphocyte ratio (NLR), and platelet-to-lymphocyte ratio (PLR) are useful biomarkers reflecting systemic inflammation and can be obtained from peripheral blood samples easily and without additional costs [6]. These markers are also associated with the development of many tumoral lesions, such as breast, gastrointestinal, oral cancer, and the survival characteristics and prognosis of these diseases [7]. The impact of the local inflammation and immune response is well known regarding cystic lesions but it is unclear what the effect of these markers is on cyst development and prognosis. Suzuki M. investigated the transporting mechanism of cystic lesions and it was found that cyst fluid contains similar ingredients with serum and lymphocytes, and segmented polymorphs existed more in cyst fluid than in serum, and they modulate the protein components of cyst fluids with active diffusion [8]. To the best of our knowledge, circulatory inflammatory cells and ratios have not been evaluated in patients with odontogenic cysts in comparison with healthy patients. Our hypothesis is that circulatory cells such as neutrophils, lymphocytes, platelets and their ratios in cyst patients could be different from healthy patients, and this could be used as a diagnostic marker in cyst patients. Therefore, the aim of this study was to compare inflammatory cells in peripheral blood tests, NLR, PLR, and MPV values in patients with odontogenic cysts and healthy subjects and the relationship of these markers between cyst type, development, and enlargement.

Material and Methods

Patient Selection

The study included 98 patients with odontogenic cysts in the maxillofacial region, who were operated under general anesthesia due to gagging reflex, dental phobia, and lack of cooperation during surgery with local anesthesia, and pre-operative blood tests were performed for the evaluation of the patients before surgery in the Oral and Maxillofacial Surgery Department. Approval from the local Ethics Committee was obtained (approval number: 2018/178). For a control, 102 patients were examined. The control group included patients who had radiological images and treated because of tooth extraction, pre-prosthetic surgery, dental implant or any other reason and had no cystic lesions in maxilla or mandible. All radiological and clinical examinations were performed by the same radiologist and surgeon. Patients with signs of infection in the maxillofacial region because of impacted teeth, pericoronitis or any other reasons were not included due to the effect on blood parameters. Patients with signs of any systemic infection, systemic diseases such as autoimmune diseases, allergies, concomitant drug use, and pregnant and lactating women were excluded from the study. Blood samples were drawn and collected into Tripotassium EDTA based tubes by hematology laboratory staff, stored at 4° C and assessed by a Sysmex K-1000 auto analyzer within 30 minutes of sampling. Platelets, white blood cells (WBC), neutrophils, lymphocyte counts and percentages were determined using a blood counter ADVIA 2120 Hematology System (Siemens AG, Eschborn, Germany).

Data collection

Data were collected from the preoperative blood tests of the patients. Clinical data about gender, age, affected region, and histopathological diagnosis of cysts were also compared.

Cone Beam Computed Tomography (CBCT) Images and Volumetric Analysis

CBCT images were obtained from the patients for detailed examination before surgery. All CBCT images were obtained with a New Tom 5G unit (QR, Verona, Italy) using standard mode with a 12 × 8 cm field of view (FOV) and a small voxel size of 250 μm3. Additionally, the CBCT unit itself modulated kilovolt (kV) and mill ampere (mA), depending on the patient. Axial slice thickness was set at 0.25 mm. All images were recorded in DICOM format and reconstructed in Simplant Pro 16 (Materialize NV, Leuven, Belgium) software. The contours of the cysts were determined for measurement of the volume of the cysts from the CBCT. Afterwards, air values were thresholded to reveal the volume value of the cysts. The drawing / delete mask and segmentation wizard techniques were used (Figure 1).

Standardization was achieved by keeping the threshold values constant in all individuals. Cyst images were examined by threshold and masking without loss of axial, coronal, and sagittal sections. The three-dimensional shaping of the cysts and the values of the volume were recorded as mm3.

Statistical Analysis

A sample size of 90 for the cyst group was calculated with power analysis. The data normality was assessed using a histogram, q-q plots, and the Shapiro-Wilk test. Variance homogeneity was examined using the Levene test. To compare the differences between groups, a two-sided independent samples t-test or the Mann-Whitney U test was applied. The Spearman test was used for correlation analysis. Moreover, receiver operating characteristics curve analysis (ROC) was applied to assess the predictive effect of markers on cyst development and volume. The area under the ROC curve was calculated with a 95% confi-dence interval. The Youden index was used to identify the optimal cut-off value. Sensitivity, specificity, as well as positive and negative predictive values were calculated with 95% confidence intervals based on the identified cut-off value [9]. Data values were expressed using mean ± standard deviation, median (1st-3rd quartiles), or frequencies (percentages). TURCOSA (Turcosa Analytics Ltd. Co., Turkey, www.turcosa.

com.tr) software was used for statistical analysis and a p-value less than 5% was considered statistically significant.

Results

A total of 200 patients with blood tests were evaluated. Ninety-eight patients were included in the cyst group (Group 1) and 102 patients were included in Group 2 as controls. 54% of all patients were women (n=108) and 46 % were men (n=92). The mean age of all patients was 27 (min: 14, max: 71) years. There was no statistical relationship between age and any of the blood parameters (p>0.05). In Group 1, 56 radicular cysts, 34 dentigerous cysts and 8 odontogenic keratocysts were observed. 54% (n=53) of the cysts were in the maxilla and 46% (n=45) were observed in the mandible. Median lymphocyte count, platelet count and MPV were statistically higher in Group 2. The optimum cut-off level for MPV was detected as 8.7 according to the ROC analysis (Sensitivity: 85, Specificity: 80) (Table 1, Figure 2).

The patients in Group 2 had an MPV value of 8.7 and higher.

Table 1. Statistical diagnostic measures of MPV in identifying cyst development

Variable	Value 95%CI
Area under ROC curve	0.86 (0.80-0.90)
Sensitivity	0.85 (0.77-0.91)
Specificity	0.80 (0.71-0.86)
Positive predictive value	0.80 (0.72-0.87)
Negative predictive value	0.85 (0.77-0.91)

CI: Confidence interval. Statistical diagnostic measures were calculated for 8.7 cut-off value (<8.7: positive)

Table 2. Results of peripheral blood tests of the patients

Variable	Group 1	Group 2	p	Total
Neutrophil count	4.66 (3.75-5.73)	4.47 (3.51-5.71)	0.898	4.59 (3.68-5.68)
Lymphocyte count	2.08 (1.63-2.58)	2.28 (1.87-2.64)	0.017	2.21 (1.80-2.61)
Platelet count	252 (217-302)	280 (247-332)	<0.001	265.5 (233-320.5)
WBC (mm^3)	7.48 (6.43-9.24)	7.86 (6.42-8.98)	0.775	7.68 (6.45-9.05)
MPV	7.6 (7.2-8.3)	9.8 (9.07-10.8)	<0.001	8.7 (7.42-10.1)
NLR(N/L)	2.12 (1.62-2.98)	1.96 (1.5-2.64)	0.129	2.06 (1.58-2.80)
PLR (P/L)	124.4 (95.9-155.6)	125.3 (103.2-150.1)	0.768	125.1 (101.1-154.1)

Variable was expressed as median (1st-3rd quartiles), Mann Whitney U Test

Table 3. Blood test results of group 1 according to the type of cyst

Variable	Radicular cyst (n=56)	Dentigerous cyst (n=34)	Odontogenic keratocyst (n=8)	P value
Neutrophil count	4.51(3.8-5.48)	4.70 (3.62-6.05)	4.47 (2.88-6.50)	0.923
Lymphocyte count	2.06 (1.65-2.56)	2.22 (1.65-2.66)	1.52 (1.34-2.11)	0.039
Platelet count	254 (219-303)	246 (225-309)	200.5 (162-293.7)	0.359
WBC (mm^3)	7.23 (6.46-9.40)	7.97 (6.63-9.31)	6.67 (4.80-8.53)	0.513
MPV	7.55 (6.95-8.20)	7.65 (7.37-8.40)	7.50 (6.90-8.70)	0.622
NLR (N/L)	2.05 (1.63-2.88)	2.41 (1.49-3.17)	2.51 (1.78-4.42)	0.399
PLR (P/L)	128.5 (95-160)	120.5 (95.7-143.3)	143.53 (114-179.4)	0.361

Variable was expressed as median (1st-3rd quartiles), Kruskal Wallis Test

Median neutrophil count and NLR were higher in Group 1 but the difference was not significant. All results of the blood tests are summarized according to patients in Table 2.

Platelet count and MPV were statistically higher in all women (n=108; p=0.014, p<0.001). MPV and NLR were different according to gender in Group 1. MPV was significantly higher in women (p=0.049) and NLR was higher in men (p=0.021). According to the type of the cyst, only lymphocyte count was significant between groups. Lymphocyte count was statistically lower in patients with odontogenic keratocysts and higher in those with dentigerous cysts (p=0.039) (Table 3).

The median cyst area was 1983,5 mm2 (min: 923,22-max: 15797) and the median cyst volume was 4663 mm3 (min: 1213-max: 48553). Cyst volume was not different according to localization of the cyst in the maxilla or mandible and type of the cyst (p=0.920, p=0.063). Spearman's correlation coefficients for neutrophil count, PLR, and cyst volume were obtained. A positive and statistically significant correlation

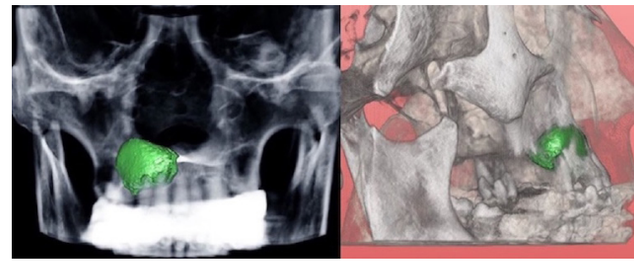

Figure 1. Determination of the contours of a radicular cyst and three dimensional thresholded image and volumetric measurement of the cyst.

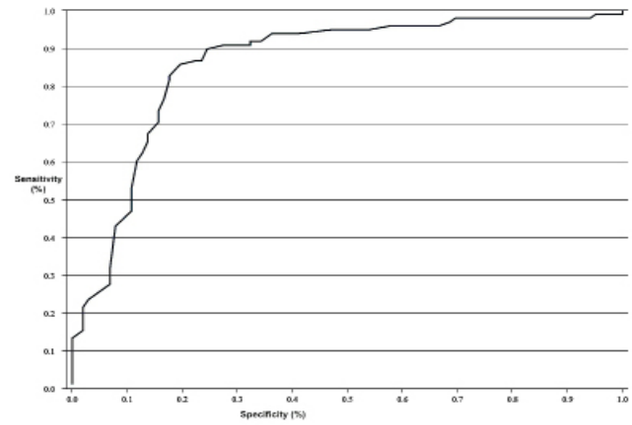

Figure 2. ROC curve indicating the predictive performance of MPV on cyst development

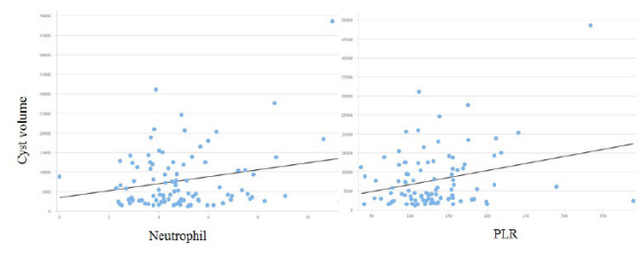

Figure 3. Correlation graphics of neutrophil count and PLR with cyst volume

between neutrophil count and PLR with cyst volume was observed (p=0.023, p=0.007) (Figure 3).

Discussion

Cystic lesions are most common treated pathologies in oral and maxillofacial surgery practice. It was reported that men are more affected by odontogenic cysts than women [1]. Radicular cysts are the most commonly seen form of these lesions [10]. The underlying mechanism of cysts is the persistent exudation of large numbers of immunocompetent cells, such as macrophages, lymphocytes, plasma cells, and leucocytes [11]. Studies in the literature stated that more lymphocytes and segmented polymorphs existed in cyst fluid than in serum and a part of the protein components of cysts are emitted as immunoglobulin by lymphocytes [8]. T- and B- lymphocytes were found a major inflammatory infiltrate of radicular cysts and have an important role in cell-mediated mechanisms in chronic inflammation [12]. Cyst development is a result of an inflammatory process in the periapical tissues and humoral and cellular immune responses play a role in the pathogenesis of these lesions [13].

Various biomarkers of humoral immune response, such as lymphocyte count, platelet count, WBC, NLR, PLR, MPV, and C reactive protein have been developed to investigate the effect of several diseases, and changes of these parameters are useful in the prognosis of many malignancies [5,13].

Neutrophils play a critical role in the progression of cancer by enhancing the proliferation, invasion and metastases via the release of cytokines. In addition, lymphocytes can eliminate cancer cells by inhibiting tumor cell proliferation and migration. High levels of lymphocyte counts were reported to be associated with a better prognosis in many types of cancer [14]. For this reason, the balance between these cells is crucial in the pathogenesis of cancer [15].

Since the relationship between chronic inflammation and carcinogenesis was reported, various studies have been focused on the role of inflammation on tumor generation, distant metastases, and prognosis [7,16]. NLR has been reported in hepatocellular carcinoma and preoperative elevated NLR was associated with a worse survival rate [17].

NLR and platelet counts are useful and PLR seems to play an important role in tumor progression. Platelets induce an epithelial/ mesenchymal transition, and the interaction between tumor cells and platelets promotes distant metastases of tumors [18].

NLR and PLR had been evaluated in patients with oral squamous cell carcinoma and it was observed that high PLR and NLR are associated with shorter survival rates [13]. Similarly, it was reported the association of high pretreatment NLR and PLR levels with poor prognosis of paranasal sinus cancer [6]. Considering the similarities between the mechanisms that are effective in the development of cysts and tumor pathogenesis, investigation of inflammatory markers in the circulation can be helpful in elucidating the effect of a humoral immune response on the pathogenesis of cysts.

Most of the studies about the effect of inflammatory cells are associated with oral cancers and metastasis of solid tumors and there are few studies about other diseases seen in the maxillofacial region. NLR was found as a useful prognostic marker for high risk of multiple recurrences in patients with adenoid cystic carcinoma [19].

It was reported in the literature that severe periodontitis was found to be associated with high platelet counts [20,21]. To the best of our knowledge, there is no study about the association between maxilla-mandibular cystic lesions and blood cells. For this reason, systemic inflammatory markers were evaluated in patients, who had radicular, dentigerous, or odontogenic keratocysts, and their relation was detected with cyst development and volume for the first time in this study. According to the results, lymphocyte count, platelet count, and MPV were statistically higher in healthy patients. These results are similar to other studies on the pathogenesis of tumors. Therefore, it can be thought that lower lymphocyte counts, which are interacting with increasing neutrophil counts, may be associated with cyst formation. Higher platelet count and MPV were also found in healthy patients in this study and MPV could be used as a diagnostic tool in cyst patients.

MPV is an indicator of platelet activation and reflects platelet production and stimulation [22]. It was reported that the size of circulating platelets is dependent on the intensity of systemic inflammation. High-grade inflammation attends a decrease of MPV, possibly due to the increased migration to the inflammatory site and high consumption of platelets at the sites of inflammation. Another statement of decreased MPV in inflammatory conditions states that the excessive production of pro-inflammatory cytokines and acute phase reactants have an impact on the size of platelets via interfering with megakaryopoiesis and the following release of small-sized platelets from the bone marrow [23]. The findings suggest that MPV was related with the inflammatory status of patients with cystic lesions. According to the ROC analysis in the present study, MPV had a high sensitivity (85%) and specificity (80%) for the evaluation of cyst pathogenesis and lower MPV values (optimum cut off: 8.7) and was found to be associated with cyst formation.

CBCT examination of cystic lesions is a feasible method for the evaluation of extension and growth in three dimensions. Kauke et al. reported that volumetric analysis of tumors by image segmentation is a precise technique [24]. They analyzed the volumetric size differentials of periapical cysts, dentigerous cysts, and keratocysts. Their results indicated that the mean volumetric extent of keratocysts is significantly higher compared to other lesions [24]. Gomez et al. found that 19% of jaw cysts exceed a volume of 10000 mm3 and they suggested a close clinic-radiologic examination of lesions with sizes exceeding a value of 3000 mm3 [17]. Higher cyst volume was seen in a keratocyst and the lowest was dentigerous cyst. Keratcyts can achieve great intraosseous volumes, most likely by multidimensional infiltration of bone [24]. According to the results of present study, it was found that volumes of cystic lesions were not different according to the type of cyst and 30% of all lesions exceed a volume of 10000 mm3 (p=0.063). The relatively low sample size for keratocysts is the limitation of present study. The main reason of the low sample size is that the patients treated under general anesthesia were included in the present study, and the patients treated in local theatre were

excluded due to the absence of the result of blood panels.

In addition, to the best of our knowledge, this is the first report, which evaluates the correlation between inflammatory markers and the volume of cysts. The authors concluded that neutrophil count and PLR have a positive correlation with cyst volume. Neutrophils may migrate to the cyst area where the inflammation is intense. This may result in an increase in circulating neutrophil production from the bone marrow and decrease in lymphocyte count. For this reason, PLR can increase due to the circulating lower lymphocyte count. It can be thought that this sequential process might be effective in the enlargement of cysts. Circulatory cells may have a potential role in this process. However, the exact mechanisms under the cyst formation and expansion are still unclear and could not be explained only by these results.

Conclusion

The present study, to the best of our knowledge, the first report in the literature, which considers the effect of circulatory inflammatory cells on the pathogenesis of jaw cysts. Odontogenic cysts are highly associated with systemic inflammatory conditions and immunological responses. Lower platelet count and MPV may play a critical role in the development of cystic lesions and neutrophils may play a role in the expansion of the cysts. Further clinical studies with more patients are needed for results that are more accurate.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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Conflict of interest

None of the authors received any type of financial support that could be considered potential conflict of interest regarding the manuscript or its submission.

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